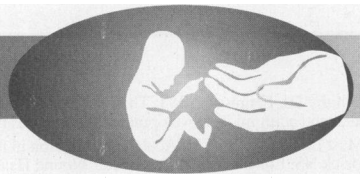

Stem Cell Transplantation

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Modern physicians desire not only to treat but to cure congenital diseases. In a wide variety of diseases, bone marrow transplantation can be the tool of final cure. The limitations and risks of this procedure have motivated researchers to search for an earlier and safer method of treatment. Special features of fetal immune systems make it possible to perform the transplantation during fetal life using fetal hematopoietic stem cells, thus avoiding many of the side effects of bone marrow transplantation in neonatal life. We review the experimental work done with animal models in this field and the human trials that have been published recently.

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Postnatal bone marrow transplantation is one possible choice in the treatment of children with a wide variety of inherited diseases. This procedure became widespread through pioneering work on the function of stem cells and the immune processes of transplantation. Initially, bone marrow transplantation was used as a therapy for diseases affecting various cell lines derived from stem cells, such as severe combined immunodeficiency disease, Wiskott-Aldrich syndrome, and the leukemias. The therapy was later proved effective in metabolic diseases such as Hurler's syndrome. Considerable limitations and risks exist with this form of therapy, however:

- An HLA-compatible donor is not always available.
- The immune response of a recipient's bone marrow must be suppressed using chemotherapy and irradiation.
- Graft-versus-host disease occurs frequently.
- The disease state may have already caused irreversible damage to the newborn.

These problems with postnatal bone marrow transplantation have motivated researchers to look for earlier and safer methods of treatment. The development of prenatal diagnostic procedures and genetic techniques has aided the search and suggested the possibility of prenatal therapy. The characteristics of fetal immunology make stem cell transplantation in utero possible using fetal stem cells, and this procedure promises to reduce the risks and disadvantages of postnatal transplantation procedures. The undeveloped immune state of the fetus eliminates the requirement for HLA compatibility and the need to actively suppress the recipient's immune response. Treating an inherited disease during gestation may offer the opportunity to correct the defect before irreversible damage occurs. The therapeutic promise of prenatal stem cell transplantation has made this procedure the object of intensive study.

Characteristics of Fetal Hematopoiesis and Immune Response

Hematopoietic stem cells originate from the yolk sac during the first few weeks of embryonic development. In the sixth and seventh weeks of human gestation, these multipotent cells migrate to the fetal liver and spleen.[1] The stem cells can differentiate into various hematopoietic and reticuloendothelial cell lineages while continuously renewing themselves. Another migration occurs during the 18th through the 20th weeks of pregnancy, when the stem cells begin to occupy the bone marrow. By the time of delivery, the only organ of hematopoiesis is the bone marrow, except in a few diseases, such as erythroblastosis fetalis, that necessitate the persistence of extramedullar hematopoiesis.

Meanwhile, the fetal immune system continues to mature. During pregnancy, the fetus is at first completely, and later partially, immunoincompetent: The inability to distinguish "self" from "foreign" causes the fetus to be immunotolerant. The placenta protects the fetus from most foreign cells and, by allowing maternal immunoglobulin G antibodies to cross this barrier, provides the fetus with immune protection. Early observations confirm that cells penetrating the placenta are accepted by the fetus and can survive long term.

A naturally occurring chimera in bovine twins due to intrauterine vascular connections was first described by Owen in 1945.[2] A follow-up study of 65 children who received transfusions in utero demonstrated circulating donor leukocytes in 5 of them a year after birth.[3] These incidents are possible because of the late development of

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fetal T lymphocytes, which are responsible for cellular immunity and the rejection of foreign grafts.[4]

Pluripotent stem cells that give rise to T lymphocytes must mature. The first wave of stem cell inflow into the thymus occurs around the eighth week of gestation.[4] Human fetal lymphocytes can proliferate in response to mitogens by the 11th to the 12th week. Postthymic T lymphocytes are capable of causing fatal graft-versus-host disease and are responsible for graft rejection; these are detected in the liver only after the 18th week of gestation.[5] These aspects of early fetal immune development make the early fetus an optimal donor and recipient.

Research in Animals

Since the observation by Lorenz and colleagues in 1951 that the bone marrow of lethally irradiated mice could be reconstituted by donor bone marrow cells,[6] remarkable efforts have been made to explain the mechanism of that therapy and to determine the advantages and risks of stem cell transplantation using various animal models. We review here selected results of studies in animals.

Mouse Models

An early observation with the in utero administration of allogeneic tissues into mouse fetuses led to the concept in 1953 of "actively acquired tolerance." Billingham and associates confirmed, with skin transplantation on the newborns injected in utero, that mice never develop, or develop to only a limited degree, immune reactivity against foreign homologous tissue to which they have been exposed sufficiently early in life.[7] They also observed that acquired tolerance is caused by a failure of the host's immune response and not by an antigenic adaptation of the grafted cells.[7]

The advantage of fetal liver cells as a source of donor stem cells was demonstrated in 1958, when Uphoff observed a reduced risk of graft-versus-host disease using fetal hematopoietic stem cells (HSCs) to reconstitute the bone marrow of lethally irradiated mice.[8] In 1966 the autosomal recessive, anemic WW mouse model was introduced. Stem cells derived from the livers of 15- to 16-day-old fetuses and transplanted into newborns cured normocytic anemia in 6 of 11 newborns.[9] Hemoglobin patterns derived from the donor erythrocytes were detected by cellulose acetate paper electrophoresis.[10] This was the first time that allogeneic fetal HSCs were used to treat a genetic disease.

In 1972 a comparative study using radioactivity incorporation showed that fetal stem cells are capable, on average, of producing larger descendant populations than are stem cells from adult bone marrow.[11] Fleischman and Mintz in 1979 were the first to use the fetus as a *recipient* of fetal HSCs.[12] They reported successful engraftment in the WW mouse model, administering fetal HSCs to the midgestational mouse placenta. A different approach, the intraperitoneal administration of HSCs, was also shown to be effective when colony-forming units were demonstrated in the spleen after fetal liver cell transplantation.[13]

Sheep Model

The first large animal model was created in 1982 by Zanjani and co-workers.[14] They transplanted adult bone marrow into sheep fetuses intravenously by placing a long-term catheter into the fetal femoral artery. This method allowed blood specimens to be obtained even before birth. Successful grafts were observed in 3 of the 5 animals surviving to birth, from 11 transplanted fetuses. The high loss rate may have been related to graft-versus-host disease. In their model, they proved that T-cell depletion of donor adult bone marrow resulted in a substantial decrease in colony formation in vitro and in reduced engraftment in vivo.[15] To avoid graft-versus-host disease, the group used fetal stem cells by the intraperitoneal route, and they had a similar success rate. Three of four fetuses were born with clear evidence of donor cell engraftment as determined by both hemoglobin type and chromosome analysis.[16]

Monkey Model

The animal models discussed earlier helped researchers to understand the development of fetal immunity and the mechanisms of HSC transplantation. Nevertheless, differences between animal and human physiology necessitated the creation of a more similar model. The results of a primate model were published in 1989 and confirmed engraftment in five of five newborn rhesus monkeys that had been transplanted in utero with fetal liver cells.[17] This study showed evidence of donor cells derived from opposite-sex fetuses in erythroid, myeloid, and lymphoid cells in recipient bone marrow.[17] Later, the data from 11 fetuses determined that the most effective time of donor cell administration was between the 60th and 80th days of gestation (term being 165 days in rhesus monkeys). This period is late enough to lower the probability of abortion and early enough to ensure a high probability of engraftment.[14]

Human Cases

Since 1968 when the first cases of bone marrow transplantation in children were reported,[18,19] hematopoietic stem cell transplantation has become a routine procedure in clinical practice. Several long-term follow-up case studies have been published that confirm the efficacy of the procedure. In 1984, Meuwissen and colleagues described the 15-year survival of a patient with Wiskott-Aldrich syndrome[20] with full T-cell, partial B-cell, and no erythroid engraftment who had received an HSC transplant in 1968.[19] With increasing study, however, limitations and risks have become clearer. Many undesirable side effects are associated with the immune status of the donor and recipient. The patients most likely to have a high engraftment rate are children with severe combined immunodeficiency, who cannot reject the donor cells. The risks of rejection and graft-versus-host disease can be reduced by using HLA-identical donors, but these can be identified for only 35% to 50% of patients. In addition, severe graft-versus-host disease may still be seen in 35% to 60% of HLA-matched patients.[21] Efforts to prevent the

disease by in vitro T-cell depletion of the donor bone marrow have reduced but have not solved the problem.

To avoid these immunologic problems while finding an accessible treatment for those children without an HLA-compatible donor, investigators selected another source of stem cells: fetal liver cells.[22,23] The only reported incidence of graft-versus-host disease occurred in a patient who received liver cells from a 16-week-old fetus.[24] This observation reinforces the preference for using cells derived from donor fetuses younger than 14 weeks' gestation.

Another problem is that by the time children are diagnosed and prepared for HSC transplantation, they may already have been damaged by the disease or by recurrent infections, irreversible mental retardation, or interventions like multiple blood transfusions.

In the past few years, approximately a dozen attempts have been made worldwide to perform stem cell transplantation prenatally. The first human in utero transplantation was reported in 1986.[25] The 17-week-old fetus received T-cell–depleted maternal bone marrow. No engraftment of Rh-negative cells was observed. During the pregnancy, several intrauterine transfusions were required. No engraftment was detectable as well in six other cases transplanted in utero with T-cell–depleted parental bone marrow.[26]

The only successful prenatal stem cell transplantations have been reported from France, using fetal stem cells.[27,28] Touraine and co-workers have treated four fetuses. Their first case was a 28-week-old fetus with the bare lymphocyte syndrome; they transplanted fetal HSCs into the umbilical vein.[27] The child was born in 1988 with evidence of engraftment—initially 10% and then 26% of lymphocytes of donor origin—and with absolute clinical reconstitution.[28] They administered fetal stem cells intravascularly in two additional cases (at the 26th and 17th weeks of gestation) and intraperitoneally in one case (at the 12th week). The indications for therapy were immunodeficiency in one case and thalassemia in two other fetuses. The follow-up studies demonstrated evidence of cells or DNA of donor origin in two newborns.[29]

Future Progress

Despite wide-ranging research and clinical work, several questions must be answered before intrauterine fetal stem cell transplantation can become a routine clinical procedure. The optimal age of donor cells is well established: before the 14th week of human gestation, before T cells appear in fetal liver. Less clear is the importance of the recipient's age at the time of fetal transplantation. From a technical point of view, the only possible approach before the 18th to 20th week is the intraperitoneal route. Research is required to determine which is more important to achieving increased engraftment: earlier treatment, using the available space in fetal bone marrow and offering decreased probability of rejection, or intravenous administration offering easier uptake of donor cells. The effectiveness of treatment may depend on the condition of the donor stem cells. New in vitro techniques are being developed to enhance stem cell numbers and effectivity.

Also offering great promise in the prenatal treatment of inherited monogeneic diseases is stem cell-mediated gene therapy.[30] Fetal stem cells derived from liver or from umbilical cord blood[31] and transfected in vitro with the desired copy of the gene in question may correct such diseases after these cells are reinjected into the fetal circulation.

Crucially, ethical considerations must regulate these manipulations, especially in human therapies. This requires the involvement not only of researchers and clinicians, but of philosophers, ethicists, educators, and lawmakers. Most important, it demands the involvement of an educated public.

Conclusions

In the past few decades, the transplantation of hematopoietic stem cells has become an important tool in treating a broad spectrum of hematopoietic and metabolic diseases. The disadvantages of postnatal bone marrow transplantation have motivated researchers and clinicians to create new procedures to overcome these hurdles.

The fetus is a proven optimal donor because:

- No T-cell depletion is required,
- No graft-versus-host disease occurs, and
- A high engraftment rate is seen.

The fetus is an optimal recipient as well because:

- No HLA matching is required,
- No immunosuppression is required,
- The probability of rejection is decreased,
- Space is available in the fetal bone marrow, and
- Early prenatal treatment can cure the condition before irreversible damage has occurred.

In selected cases when the diagnosis can be established early enough in gestation and the disease is life-threatening, intrauterine fetal stem cell transplantation is a feasible treatment of congenital disease in the hope of a long-term cure.

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